

**LISTING OF ALL CLAIMS AMENDED RELATIVE TO ORIGINAL PATENT**  
**(CUMUMLATIVE TO DATE)**

1. (amended) A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, [wherein said antigen has the following characteristics:  
(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;  
(b) said antigen is not detectable on normal colon cancer free human tissues;  
(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;  
(d) said antigen is specifically immunogenic in humans; and  
(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity] which is murine monoclonal antibody 33.28 as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.
2. (amended) [An] A purified antibody [according to claim 1 which is mouse monoclonal antibody 33.28 (ATCC HB-12315) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 3328] which competitively inhibits binding of the antibody of claim 1 to the human colon carcinoma - associated protein antigen.
3. (amended) An antibody according to claim 2 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 61.1 kilodaltons as measured by gradient polyacrylamide gel electrophoresis.
4. (amended) [An antibody according claim 1] A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, which is mouse monoclonal antibody 31.1, as produced by hybridoma PCA 31.1 [(ATCC HB-12314)] , deposited with the American Type Culture Collection and assigned accession number PTA-2497[

or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 31.1].

5. (amended) [An] A purified antibody [according to claim 4 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 72 kilodaltons] which competitively inhibits binding of the antibody of claim 4 to the human colon carcinoma - associated protein antigen.

6. (amended) An antibody according to claim [2] § wherein said colon carcinoma-associated antigen is a glycoprotein, the protein component having a molecular weight of [61.1] about 72 kilodaltons kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

7. (amended) An antibody according to claim 1, 2, 4 or 5 immobilized on a solid phase.

8. (amended) An antibody according to claim 1, 2, 4 or 5 which is detectably labeled.

10. (amended) An antibody according to claim 1, 2, 4 or 5 conjugated to a cytotoxic radionuclide.

11. (amended) An antibody according to claim 1, 2, 4 or 5 conjugated to a cytotoxic drug.

12. (amended) An antibody according to claim 1, 2, 4 or 5 conjugated to a cytotoxic protein.

[16. A monoclonal antibody against the monoclonal antibody of claim 1.]

[17. A monoclonal antibody against the monoclonal antibody of claim 2.]

[18. A monoclonal antibody against the monoclonal antibody of claim 3. ]

[19. A monoclonal antibody against the monoclonal antibody of claim 4. ]

[20. A monoclonal antibody against the monoclonal antibody of claim 5. ]

[21. A monoclonal antibody against the monoclonal antibody of claim 6. ]

22. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 33.28 [(ATCC HB-12315)] as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413, in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim 1 or claim 2; and
- (b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma - associated protein antigen.

23. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 31.1 [(ATCC HB-12314)] as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497, in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim [1] 4 or claim 5; and
- (b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma - associated protein antigen.

24. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon cancer;
- (b) contacting the specimen with monoclonal antibody 33.28 [(ATCC HB-12315)] as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;

- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

25. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having colon[-]carcinoma;
- (b) contacting the specimen with mouse monoclonal antibody 31.1 [(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex.

28. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 31.1[(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

29. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

30. (amended) A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, [wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,]

said kit comprising a first container adapted to contain an antibody according to claim 2 or 5 [to said antigen or an active component thereof], and a second container adapted to contain a second antibody to said antigen [or an active component thereof], said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

33. (amended) A kit according to claim [30] 32 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

34. (amended) A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, [wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,]

said kit comprising a first container adapted to contain monoclonal antibody 31.1 [(ATCC HB-12314)] as produced by hybridoma PCA 31.1, deposited with the

American Type Culture Collection and assigned accession number PTA-2497, to said antigen and a second container adapted to contain a second antibody to said antigen [or an active component thereof], said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

36. (amended) A kit according to claim [32] 34 wherein the reporter molecule is an enzyme.

37. (amended) A kit according to claim [33] 36 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

38. (amended) A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, [wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,]

said kit comprising a first container adapted to contain monoclonal antibody 33.28, as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413 [(ATCC HB-12315)] to said antigen and a second container adapted to contain a second antibody to said antigen [or an active component thereof], said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

41. (amended) A kit according to claim [38] 40 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

[42. The monoclonal antibody of claim 1 which is a chimeric antibody.]

43. (amended) [The] A chimeric antibody [according to claim 42] which is a chimeric mouse/human antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316()].

44. (amended) The chimeric antibody according to claim [42] 43 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of 72 [kilodalton] kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

45. (amended) A composition comprising the chimeric antibody according to claim [42] 43 in combination with a pharmaceutically acceptable carrier.

[46. A monoclonal antibody against the chimeric antibody of claim 42.]

47. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to the mouse/human chimeric antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316()] of claim 42] in a sample comprising:

- (a) contacting said sample with the Chi #1 antibody [according to claim 42]; and
- (b) detecting said antigen by detecting the binding of said antibody to the [purified] colon carcinoma-associated protein antigen.

48. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon carcinoma;
- (b) contacting the specimen with a chimeric antibody [which binds to an antigen] according to claim [1] 43;
- (c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

49. (amended) A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon carcinoma;

(b) contacting the specimen with mouse/human chimeric antibody which binds to an antigen which binds to mouse/human chimeric antibody Chi #1 [(ATCC) as produced by the cell line deposited with the American Type Culture Collection and assigned accession number CRL-12316()];

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

51. (new) A purified antibody which is raised against an immunopurified human colon carcinoma associated antigen that is specifically bound by monoclonal antibody 31.1, as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497.

52.(new) The antibody of claim 51 which is a monoclonal antibody.

53. (new) An antibody which is raised against a purified human colon carcinoma associated antigen that is specifically bound by monoclonal antibody 33.28, as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.

54. (new) The antibody of claim 53 which is a monoclonal antibody.



chain variable region gene to the exon of the human gamma chain constant region gene using the polymerase chain reaction. Subsequently, the 31.1 chimeric gene was cloned into a retroviral expression vector pLpntCXII and transfected into the packaging cell line PA317. The transfected cells (PA317H) were cultivated with another packaging cell line PA317L, which contained an irrelevant mouse/human chimeric light chain gene in retroviral expression vector pLneoCXII, and SP2/0-Ag14 cells. The transduced SP2/0-Ag14 cells yielded a complete chimeric antibody, Chi #1 which reacted with horseradish peroxidase-conjugated IgG of goat anti-human IgG Fc in ELISA analyses, which indicated that the constant region of Chi #1 was human. Cytofluorometry analysis indicated that Chi #1 stained human colorectal carcinoma cell lines HT-29 and LS174T but not a human lung carcinoma cell line A-427. Antibody-dependent cell-mediated cytotoxicity (ADCC) assay indicated that Chi #1 lysed LS174T cells. These results show that Chi #1 retained the antigen-binding specificity of the parental 31.1 mouse monoclonal antibody, suggesting the usefulness of this chimeric antibody in ascertaining prognosis of colon carcinoma.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

What is claimed is:

1. A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, wherein said antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity.

2. An antibody according to claim 1 which is mouse monoclonal antibody 33.28 (ATCC HB-12315) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 3328.

3. An antibody according to claim 2 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 61.1 kilodaltons.

4. An antibody according claim 1 which is mouse monoclonal antibody 31.1 (ATCC HB-12314) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 31.1.

5. An antibody according to claim 4 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 72 kilodaltons.

6. An antibody according to claim 2 wherein said colon carcinoma-associated antigen is a glycoprotein, the protein component having a molecular weight of 61.1 kilodaltons.

7. An antibody according to claim 1 immobilized on a solid phase.

8. An antibody according to claim 1 which is detectably labelled.

9. An antibody according to claim 8 wherein said detectable label is a radiolabel.

10. An antibody according to claim 1 conjugated to a cytotoxic radionuclide.

11. An antibody according to claim 1 conjugated to a cytotoxic drug.

12. An antibody according to claim 1 conjugated to a cytotoxic protein.

13. A composition comprising an antibody according to claim 10 in combination with a pharmaceutically acceptable carrier.

14. A composition comprising an antibody according to claim 11 in combination with a pharmaceutically acceptable carrier.

15. A composition comprising an antibody according to claim 12 in combination with a pharmaceutically acceptable carrier.

16. A monoclonal antibody against the monoclonal antibody of claim 1.

17. A monoclonal antibody against the monoclonal antibody of claim 2.

18. A monoclonal antibody against the monoclonal antibody of claim 3.

19. A monoclonal antibody against the monoclonal antibody of claim 4.

20. A monoclonal antibody against the monoclonal antibody of claim 5.

21. A monoclonal antibody against the monoclonal antibody of claim 6.

22. An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 33.28 (ATCC HB-12315) in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim 1; and
- (b) detecting said antigen by detecting the binding of the antibody to the purified colon carcinoma associated protein antigen.

23. An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 31.1 (ATCC HB-12314) in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim 1; and
- (b) detecting said antigen by detecting the binding of the antibody to the purified colon carcinoma associated protein antigen.

24. A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon cancer;
- (b) contacting the specimen with monoclonal antibody 33.28 (ATCC HB-12315);
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

25. A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having colon-carcinoma;
- (b) contacting the specimen with mouse monoclonal antibody 31.1 (ATCC HB-12314);
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex.

26. A method according to claim 24 wherein the stain is an avidin-biotin immunoperoxidase stain.

27. A method according to claim 25 wherein the stain is an avidin-biotin immunoperoxidase stain.

28. A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 31.1 (ATCC HB-12314);
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

29. A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 33.28 (ATCC HB-12315);
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

30. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain an antibody to said antigen or an active component thereof, and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

31. A kit according to claim 30 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.

32. A kit according to claim 30 wherein the reporter molecule is an enzyme.

33. A kit according to claim 30 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

34. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain monoclonal antibody 31.1 (ATCC HB-12314) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

35. A kit according to claim 34 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.

36. A kit according to claim 32 wherein the reporter molecule is an enzyme.

37. A kit according to claim 33 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

38. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
- (b) said antigen is not detectable on normal colon cancer free human tissues;
- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
- (d) said antigen is specifically immunogenic in humans; and
- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain monoclonal antibody 33.28 (ATCC HB-12315) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

39. A kit according to claim 38 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.

40. A kit according to claim 38 wherein the reporter molecule is an enzyme.

41. A kit according to claim 38 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

42. The monoclonal antibody of claim 1 which is a chimeric antibody.

43. The chimeric antibody according to claim 42 which is a chimeric mouse/human antibody Chi #1 (ATCC CRL-12316).

44. The chimeric antibody according to claim 42 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of 72 kilodalton.

45. A composition comprising the chimeric antibody according to claim 42 in combination with a pharmaceutically acceptable carrier.

46. A monoclonal antibody against the chimeric antibody of claim 42.

47. An immunoassay for detecting a colon carcinoma-associated antigen which binds to the mouse/human chi-

meric antibody Chi #1 (ATCC CRL-12316) of claim 42 in a sample comprising:

(a) contacting said sample with the antibody according to claim 42; and

(b) detecting said antigen by detecting the binding of said antibody to the purified colon carcinoma associated protein antigen.

48. A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon carcinoma;

(b) contacting the specimen with a chimeric antibody which binds to an antigen according to claim 1;

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

49. A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon carcinoma;

(b) contacting the specimen with mouse/human chimeric antibody which binds to an antigen which binds to mouse/human chimeric antibody Chi #1 (ATCC CRL-12316);

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

50. A kit for the immunohistochemical detection of colon carcinoma comprising:

(a) mouse/human chimeric antibody Chi #1 (ATCC CRL-12316);

(b) reagents for immunoperoxidase and secondary antibody;

immunoperoxidase; and

(d) colorizing reagents.

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